Rec'd PCT/PTO 31 MAR 1998

FORM PTO-1390 (REV 10-94)

U S DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE

TRANSMITTAL LETTER TO THE UNITED STATES DESIGNATED/ELECTED OFFICE (DO/EO/US) CONCERNING A FILING UNDER 35 U.S.C. 371

ATTORNEY'S DOCKET NUMBER

3164.98USWO

09/ 051034

U S APPLICATION NO (If known, see 37 C F R 1 5)

Unknown

INTERNATIONAL APPLICATION NO.	INTERNATIONAL FILING DATE	PRIORITY DATE CLAIMED
PCT/AU97/00492	August 1, 1997	August 2, 1996

TITLE OF INVENTION

IMPROVED NUCLEIC ACIDS ENCODING A CHIMERIC GLYCOSYLTRANSFERASE

APPLICANT(S) FOR DO/EO/US

Ian Farquhar Campbell MCKENZIE; Mauro Sergio SANDRIN

Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information:

1. [X] This is a **FIRST** submission of items concerning a filing under 35 U.S.C. 371.
2. [ ] This is a **SECOND** or **SUBSEQUENT** submission of items concerning a filing under 35 U.S.C. 371.
3. [X] This express request to begin national examination procedures (35 U.S.C. 371(f)) at any time rather than delay examination until the expiration of the applicable time limit set in 35 U.S.C. 371(b) and PCT Articles 22 and 39(1).
4. [ ] A proper Demand for International Preliminary Examination was made by the 19th month from the earliest claimed priority date.
5. [X] A copy of the International Application as filed (35 U.S.C. 371(c)(2))
   a. [X] is transmitted herewith (required only if not transmitted by the International Bureau).
   b. [X] has been transmitted by the International Bureau.
   c. [ ] is not required, as the application was filed in the United States Receiving Office (RO/US)
6. [ ] A translation of the International Application into English (35 U.S.C. 371(c)(2)).
7. [X] Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. 371(c)(3))
   a. [ ] are transmitted herewith (required only if not transmitted by the International Bureau).
   b. [ ] have been transmitted by the International Bureau.
   c. [X] have not been made; however, the time limit for making such amendments has NOT expired.
   d. [ ] have not been made and will not be made.
8. [ ] A translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 371(c)(3)).
9. [X] A signed oath or declaration of the inventor(s) (35 U.S.C. 371 (c)(4)).
10. [ ] A translation of the annexes to the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371(c)(5)).

Items 11. to 16. below concern document(s) or information included:

11. [X] An Information Disclosure Statement under 37 CFR 1.97 and 1.98.
12. [X] An assignment document for recording. A separate cover sheet in compliance with 37 CFR 3.28 and 3.31 is included.
13. [X] A FIRST preliminary amendment.
    [ ] A SECOND of SUBSEQUENT preliminary amendment.
14. [ ] A substitute specification.
15. [ ] A change of power of attorney and/or address letter.
16. [X] Other items or information: PTO Form 1449; Communication Re: Inventorship; International Search Report

U.S. APPLICATION NO (If known, see 37 C F R 1 5)	INTERNATIONAL APPLICATION NO PCT/AU97/00492	ATTORNEY'S DOCKET NUMBER 3164.98USWO

17. [X] The following fees are submitted:

BASIC NATIONAL FEE (37 CFR 1.492(a) (1)-(5)):

Search Report has been prepared by the EPO or JPO **$930.00**

International preliminary examination fee paid to USPTO (37 CFR 1.492(a)(1)) **$720.00**

No international preliminary examination fee paid to USPTO (37 CFR 1.482) but international search fee paid to USPTO (37 CFR 1.445(a)(2)) **$790.00**

Neither international preliminary examination fee (37 CFR 1.482) nor international search fee (37 CFR 1.445(a)(3)) paid to USPTO **$1,070.00**

International preliminary examination fee paid to USPTO (37 CFR 1.482) and all claims satisfied provisions of PCT Article 33(2)-(4) **$98.00**

				CALCULATIONS	PTO USE ONLY
ENTER APPROPRIATE BASIC FEE AMOUNT =				**$535.00**	
Surcharge of **$130.00** for furnishing the oath or declaration later than [ ] 20 [ ] 30 months from the earliest claimed priority date (37 CFR 1.492(e)).				**$**	
CLAIMS	NUMBER FILED	NUMBER EXTRA	RATE		
Total claims	25 -20 =	5	X **$11.00**	**$55.00**	
Independent claims	4 -3 =	1	X **$41.00**	**$41.00**	
MULTIPLE DEPENDENT CLAIM(S) (if applicable)			+ **$270.00**	**$**	
TOTAL OF ABOVE CALCULATIONS =				**$631.00**	
Reduction by 1/2 for filing by small entity, if applicable. Verified Small Entity Statement must also be filed (Note 37 CFR 1.9, 1.27, 1.28).				**$**	
SUBTOTAL =				**$631.00**	
Processing fee of **$130.00** for furnishing the English translation later than [ ] 20 [ ] 30 months from the earliest claimed priority date (37 CFR 1.492(f). +				**$**	
TOTAL NATIONAL FEE =				**$631.00**	
Fee for recording the enclosed assignment (37 CFR 1.21(h)). The assignment must be accompanied by an appropriate cover sheet (37 CFR 3.28, 3.31). $40.00 per property +				**$40.00**	
TOTAL FEES ENCLOSED =				**$671.00**	
				Amount to be: refunded	**$**
				charged	**$**

a. [X] Checks in the amount of $ 631.00 and $40.00 to cover the above fees are enclosed.

b. [ ] Please charge my Deposit Account No. ________ in the amount of $ ________ to cover the above fees. A duplicate copy of this sheet is enclosed.

c. [X] The Commissioner is hereby authorized to charge any additional fees which may be required, or credit any overpayment to Deposit Account No. 13-2725. A duplicate copy of this sheet is enclosed.

NOTE: Where an appropriate time limit under 37 CFR 1.494 or 1.495 has not been met, a petition to revive (37 CFR 1.137(a) or (b)) must be filed and granted to restore the application to pending status.

SEND ALL CORRESPONDENCE TO
Michael L. Mau
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SIGNATURE

Michael L. Mau
NAME

30,087
REGISTRATION NUMBER

Rec'd PCT/PTO 31 MAR 1998

09/051034

S/N Unknown PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant:	McKENZIE et al.	Examiner:	Unknown
Serial No.:	Unknown	Group Art Unit:	Unknown
Filed:	Intl Filing Date August 1, 1997	Docket No.:	3164.98USWO
Title:	IMPROVED NUCLEIC ACIDS ENCODING A CHIMERIC GLYCOSYLTRANSFERASE		

CERTIFICATE UNDER 37 CFR 1.10

'Express Mail' mailing label number: EM422712114US

Date of Deposit: March 31, 1998

I hereby certify that this correspondence is being deposited with the United States Postal Service 'Express Mail Post Office To Addressee' service under 37 CFR 1.10 on the date indicated above and is addressed to the Assistant Commissioner for Patents, Washington, D.C. 20231.

By: ____________________

Name: William Smith

PRELIMINARY AMENDMENT

Box PCT
Assistant Commissioner for Patents
Washington, D. C. 20231

Sir:

In connection with the above-identified application filed herewith, please enter the following preliminary amendment:

IN THE ABSTRACT

Insert the attached Abstract page into the application as the last page thereof.

IN THE SPECIFICATION

A courtesy copy of the present specification is enclosed herewith, but the World Intellectual Property Office (WIPO) copy should be relied upon if it is already in the U.S. Patent Office.

IN THE CLAIMS

In claim 3, line 1, delete "or claim 2".

In claim 4, line 1 to 2, delete "any one of claims 1 to 3" and insert --claim 1--.

In claim 5, line 1 to 2, delete "any one of claims 1 to 4" and insert --claim 1--.

In claim 6, line 1 to 2, delete "any one of claims 1 to 5" and insert --claim 1--.

In claim 7, line 1 to 2, delete "any one of claims 1 to 6" and insert --claim 1--.

In claim 8, line 1 to 2, delete "any one of claims 1 to 7" and insert --claim 1--.

In claim 9, line 1 to 2, delete "any one of claims 1 to 8" and insert --claim 1--.

In claim 11, line 1 to 2, delete "any one of claims 1 to 10" and insert --claim 1--.

In claim 12, line 2, delete "any one of claims 1 to 11" and insert --claim 1--.

In claim 14, line 1, delete "or claim 13".

In claim 17, line 2, delete "any one of claims 1 to 11" and insert --claim 1--.

In claim 22, line 2 to 3, delete "any one of claims 1 to 11" and insert --claim 1--.

In claim 23, line 2, delete "any one of claims 1 to 11" and insert --claim 1--.

In claim 25, line 2, delete "or claim 24".

REMARKS

A new abstract page is supplied to conform to that appearing on the publication page of the WIPO application, but the new Abstract is typed on a separate page as required by U.S. practice.

The above preliminary amendment is made to remove multiple dependencies from claims 1 to 9, 11 to 12, 14, 17, 22 to 23 and 25.

Applicant respectfully requests that the preliminary amendment described herein be entered into the record prior to examination and consideration of the above-identified application.

If a telephone conference would be helpful in resolving any issues concerning this communication, please contact Applicant's primary attorney-of record, Michael L. Mau (Reg. No. 30,422), at (612) 336–4727.

Respectfully submitted,

McKENZIE et al.

By their attorneys,

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Dated: March 31, 1998

By______________________
Michael L. Mau
Reg. No. 30,087

MLM/sef

PCT/AU97/00492
Docket: 3164.98USWO

Abstract

The invention relates to nucleic acids which encode glycosyltransferase and are useful in producing cells and organs from one species which may be used for transplantation into a recipient of another species. It also relates to the production of nucleic acids which, when present in cells of a transplanted organ, result in reduced levels of antibody recognition of the transplanted organ.

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[illegible] that this paper or fee is being deposited [illegible] States Postal Service "Express Mail Post [illegible]" service under 37 CFR 1.10 on the [illegible] above and is addressed to the Commis- [illegible] and Trademarks, Washington, D.C. 20231

William Smith
Printed name

Signature

09/051034

IMPROVED NUCLEIC ACIDS ENCODING A CHIMERIC GLYCOSYLTRANSFERASE

Field of the Invention

The present invention relates to nucleic acids which encode glycosyltransferase and are useful in producing cells and organs from one species which may be used for transplantation into a recipient of another species. Specifically the invention concerns production of nucleic acids which, when present in cells of a transplanted organ, result in reduced levels of antibody recognition of the transplanted organ.

Background of the Invention

The transplantation of organs is now practicable, due to major advances in surgical and other techniques. However, availability of suitable human organs for transplantation is a significant problem. Demand outstrips supply. This has caused researchers to investigate the possibility of using non-human organs for transplantation.

Xenotransplantation is the transplantation of organs from one species to a recipient of a different species. Rejection of the transplant in such cases is a particular problem, especially where the donor species is more distantly related, such as donor organs from pigs and sheep to human recipients. Vascular organs present a special difficulty because of hyperacute rejection (HAR).

HAR occurs when the complement cascade in the recipient is initiated by binding of antibodies to donor endothelial cells.

Previous attempts to prevent HAR have focused on two strategies : modifying the immune system of the host by inhibition of systemic complement formation (1,2), and antibody depletion (3,4). Both strategies have been shown to prolong xenograft survival temporarily. However, these methodologies are therapeutically unattractive in that they are clinically impractical, and would require chronic

immunosuppressive treatments. Therefore, recent efforts to inhibit HAR have focused on genetically modifying the donor xenograft. One such strategy has been to achieve high-level expression of species-restricted human complement inhibitory proteins in vascularized pig organs via transgenic engineering (5-7). This strategy has proven to be useful in that it has resulted in the prolonged survival of porcine tissues following antibody and serum challenge (5,6). Although increased survival of the transgenic tissues was observed, long-term graft survival was not achieved (6). As observed in these experiments and also with systemic complement depletion, organ failure appears to be related to an acute antibody-dependent vasculitis (1,5).

In addition to strategies aimed at blocking complement activation on the vascular endothelial cell surface of the xenograft, recent attention has focused on identification of the predominant xenogeneic epitope recognised by high-titre human natural antibodies. It is now accepted that the terminal galactosyl residue, Gal-α (1,3)-Gal, is the dominant xenogeneic epitope (8-15). This epitope is absent in Old World primates and humans because the α(1,3)-galactosyltransferase (gal-transferase or GT) is non-functional in these species. DNA sequence comparison of the human gene to α(1,3)-galactosyltransferase genes from the mouse (16,17), ox (18), and pig (12) revealed that the human gene contained two frameshift mutations, resulting in a non-functional pseudogene (20,21). Consequently, humans and Old World primates have pre-existing high-titre antibodies directed at this Gal-α(1,3)-Gal moiety as the dominant xenogeneic epitope.

One strategy developed was effective to stably reduce the expression of the predominant Gal-α(1,3)-Gal epitope. This strategy took advantage of an intracellular competition between the gal-transferase and α(1,2)-fucosyltransferase (H-transferase) for a common acceptor substrate. The gal-transferase catalyses the transfer of a

terminal galactose moiety to an N-acetyl lactosamine acceptor substrate, resulting in the formation of the terminal Gal-α(1,3)-Gal epitope. Conversely, H-transferase catalyses the transfer of a fucosyl residue to the N-acetyl lactosamine acceptor substrate, and generates a fucosylated N-acetyl lactosamine (H-antigen, *i.e.*, the O blood group antigen), a glycosidic structure that is universally tolerated. Although it was reported that expression of human H-transferase transfected cells resulted in high level expression of the non-antigenic H-epitope and significantly reduced the expression of the Gal-α(1,3)-Gal xenoepitope, there are still significant levels of Gal-α(1,3)-Gal epitope present on such cells.

Summary of the Invention

In view of the foregoing, it is an object of the present invention to further reduce levels of undesirable epitopes in cells, tissues and organs which may be used in transplantation.

In work leading up to the invention the inventors surprisingly discovered that the activity of H transferase may be further increased by making a nucleic acid which encodes a H transferase catalytic domain but is anchored in the cell at a location where it is better able to compete for substrate with gal transferase. Although work by the inventors focused on a chimeric H transferase, other glycosyltransferase enzymes may also be produced in accordance with the invention.

Accordingly, in a first aspect the invention provides a nucleic acid encoding a chimeric enzyme, wherein said chimeric enzyme comprises a catalytic domain of a first glycosyltransferase and a localisation signal of a second glycosyltransferase, whereby when said nucleic acid is expressed in a cell said chimeric enzyme is located in an area of the cell where it is able to compete for substrate with a second glycosyltransferase, resulting in reduced levels of a product from said second

glycosyltransferase.

Preferably the nucleic acid is in an isolated form; that is the nucleic acid is at least partly purified from other nucleic acids or proteins.

Preferably the nucleic acid comprises the correct sequences for expression, more preferably for expression in a eukaryotic cell. The nucleic acid may be present on any suitable eukaryotic expression vector such as pcDNA (Invitrogen). The nucleic acid may also be present on other vehicles whether suitable for eukaryotes or not, such as plasmids, phages and the like.

Preferably the catalytic domain of the first glycosyltransferase is derived from H transferase, secretor sialyltransferase, a galactosyl sulphating enzyme or a phosphorylating enzyme.

The nucleic acid sequence encoding the catalytic domain may be derived from, or similar to a glycosyltransferase from any species. Preferably said species is a mammalian species such as human or other primate species, including Old World monkeys, or other mammals such as ungulates (for example pigs, sheep, goats, cows, horses, deer, camels) or dogs, mice, rats and rabbits. The term "similar to" means that the nucleic acid is at least partly homologous to the glycocyltransferase genes described above. The term also extends to fragments of and mutants, variants and derivatives of the catalytic domain whether naturally occurring or man made.

Preferably the localisation signal is derived from a glycosyltransferase which produces glycosylation patterns which are recognised as foreign by a transplant recipient. More preferably the localisation signal is derived from $\alpha(1,3)$ galactosyltransferase. The effect of this is to downregulate the level of Gal-$\alpha(1,3)$-Gal produced in a cell when the nucleic acid is expressed by the cell.

The nucleic acid sequence encoding the localisation signal may be derived from any species such as

those described above. Preferably it is derived from the same species as the cell which the nucleic acid is intended to transform i.e., if pig cells are to be transformed, preferably the localization signal is derived from pig.

More preferably the nucleic acid comprises a nucleic acid sequence encoding the catalytic domain of H transferase and a nucleic acid sequence encoding a localisation signal from Gal transferase. Still more preferably both nucleic acid sequences are derived from pigs. Even more preferably the nucleic acid encodes gtHT described herein.

The term "nucleic acid" refers to any nucleic acid comprising natural or synthetic purines and pyrimidines. The nucleic acid may be DNA or RNA, single or double stranded or covalently closed circular.

The term "catalytic domain" of the chimeric enzyme refers to the amino acid sequences necessary for the enzyme to function catalytically. This comprises one or more contiguous or non-contiguous amino acid sequences. Other non-catalytically active portions also may be included in the chimeric enzyme.

The term "glycosyltransferase" refers to a polypeptide with an ability to move carbohydrates from one molecule to another.

The term "derived from" means that the catalytic domain is based on, or is similar, to that of a native enzyme. The nucleic acid sequence encoding the catalytic domain is not necessarily directly derived from the native gene. The nucleic acid sequence may be made by polymerase chain reaction (PCR), constructed *de novo* or cloned.

The term "localisation signal" refers to the amino acid sequence of a glycosyltransferase which is responsible for anchoring it in location within the cell. Generally localisation signals comprise amino terminal "tails" of the enzyme. The localisation signals are derived from a second glycosyltransferase, the activity of which it is desired to minimise. The localisation of a

catalytic domain of a first enzyme in the same area as the second glycosyltransferase means that the substrate reaching that area is likely to be acted on by the catalytic domain of the first enzyme, enabling the amount of substrate catalysed by the second enzyme to be reduced.

The term "area of the cell" refers to a region, compartment or organelle of the cell. Preferably the area of the cell is a secretory organelle such as the Golgi apparatus.

In another aspect the invention provides an isolated nucleic acid molecule encoding a localisation signal of a glycosyltransferase. Preferably the signal encoded comprises an amino terminus of said molecule; more preferably it is the amino terminus of gal transferase. The gal transferase may be derived from or based on a gal transferase from any mammalian species, such as those described above. Particularly preferred sequences are those derived from pig, mouse or cattle.

In another aspect the invention relates to a method of producing a nucleic acid encoding a chimeric enzyme, said enzyme comprising a catalytic domain of a first glycosyltransferase and a localisation signal of a second glycosyltransferase whereby when said nucleic acid is expressed in a cell said chimeric enzyme is located in an area of the cell where it is able to compete for substrate with a second glycosyltransferase said method comprising operably linking a nucleic acid sequence encoding a catalytic domain from a first glycosyltransferase to a nucleic acid sequence encoding a localisation signal of a second glycosyltransferase.

The term "operably linking" means that the nucleic acid sequences are ligated such that a functional protein is able to be transcribed and translated.

Those skilled in the art will be aware of various techniques for producing the nucleic acid. Standard techniques such as those described in Sambrook et al may be employed.

Preferably the nucleic acid sequences are the preferred sequences described above.

In another aspect the invention provides a method of reducing the level of a carbohydrate exhibited on the surface of a cell, said method comprising causing a nucleic acid to be expressed in said cell wherein said nucleic acid encodes a chimeric enzyme which comprises a catalytic domain of a first glycosyltransferase and a localisation signal of a second glycosyltransferase, whereby said chimeric enzyme is located in an area of the cell where it is able to compete for substrate with said second glycosyltransferase, and wherein said second glycosyltransferase is capable of producing said carbohydrate.

The term "reducing the level of a carbohydrate" refers to lowering, minimising, or in some cases, ablating the amount of carbohydrate displayed on the surface of the cell. Preferably said carbohydrate is capable of stimulating recognition of the cell as "non-self" by the immune system of an animal. The reduction of such a carbohydrate therefore renders the cell, or an organ composed of said cells, more acceptable to the immune system of a recipient animal in a transplant situation or gene therapy situation.

The term "causing a nucleic acid to be expressed" means that the nucleic acid is introduced into the cell (i.e. by transformation/transfection or other suitable means) and contains appropriate signals to allow expression in the cells.

The cell may be any suitable cell, preferably mammalian, such as that of a New World monkey, ungulate (pig, sheep, goat, cow, horse, deer, camel, etc.) or other species such as dogs.

In another aspect the invention provides a method of producing a cell from one species (the donor) which is immunologically acceptable to another species (the recipient) by reducing levels of carbohydrate on said cell

which cause it to be recognised as non-self by the other species, said method comprising causing a nucleic acid to be expressed in said cell wherein said nucleic acid encodes a chimeric enzyme which comprises a catalytic domain of a first glycosyltransferase and a localisation signal of a second glycosyltransferase, whereby said chimeric enzyme is located in an area of the cell where it is able to compete for substrate with said second glycosyltransferase, and wherein said second glycosyltransferase is capable of producing said carbohydrate.

The term "immunologically acceptable" refers to producing a cell, or an organ made up of numbers of the cell, which does not cause the same degree of immunological reaction in the recipient species as a native cell from the donor species. Thus the cell may cause a lessened immunological reaction, only requiring low levels of immunosuppressive therapy to maintain such a transplanted organ or no immunosuppression therapy.

The cell may be from any of the species mentioned above. Preferably the cell is from a New World primate or a pig. More preferably the cell is from a pig.

The invention extends to cells produced by the above method and also to organs comprising the cells.

The invention further extends to non-human transgenic animals harbouring the nucleic acid of the invention. Preferably the species is a human, ape or Old World monkey.

The invention also extends to the proteins produced by the nucleic acid. Preferably the proteins are in an isolated form.

In another aspect the invention provides an expression unit which expresses the nucleic acid of the invention, resulting in a cell which is immunologically acceptable to an animal having reduced levels of a carbohydrate on its surface, which carbohydrate is recognised as non-self by said species. In a preferred embodiment, the expression unit is a retroviral packaging

cell, cassette, a retroviral construct or retroviral producer cell.

Preferably the species is a human, ape or Old World monkey.

The retroviral packaging cells or retroviral producer cells may be cells of any animal origin where it is desired to reduce the level of carbohydrates on its surface to make it more immunologically acceptable to a host. Such cells may be derived from mammals such as canine, rodent or ruminant species and the like.

The retroviral packaging and/or producer cells may be used in applications such as gene therapy. General methods involving use of such cells are described in PCT/US95/07554 and the references discussed therein.

The invention also extends to a method of producing a retroviral packaging cell or a retroviral producer cell having reduced levels of a carbohydrate on its surface wherein the carbohydrate is recognised as non-self by a species, comprising transforming/transfecting a retroviral packaging cell or a retroviral producer cell with the nucleic acid of the invention under conditions such that the chimeric enzyme is produced.

Brief Description of the Drawings

Figure 1 **Schematic diagram of normal and chimeric glycosyltransferases**

The diagram shows normal glycosyltransferases porcine α(1,3)galactosyltransferase (GT) and human α(1,2)fucosyltransferase (HT), and chimeric transferases ht-GT in which the cytoplasmic domain of GT has been completely replaced by the cytoplasmic domain of HT, and gt-HT in which the cytoplasmic domain of HT has been entirely replaced by the cytoplasmic domain of GT. The protein domains depicted are cytoplasmic domain CYTO, transmembrane domain TM, stem region STEM, catalytic domain CATALYTIC. The numbers refer to the amino acid sequence of

the corresponding normal transferase.

Figure 2 Cell surface staining of COS cells transfected with normal and chimeric transferases

Cells were transfected with normal GT or HT or with chimeric transferases gt-HT or ht-GT and 48h later were stained with FITC-labelled lectin IB4 or UEAI. Positive-staining cells were visualised and counted by fluorescence microscopy. Results are from at least three replicates and values are +/- SEM.

Figure 3. RNA analysis of transfected COS cells

Northern blots were performed on total RNA prepared from COS cells transfected: Mock, mock-transfected; GT, transfected with wild-type GT; GT1-6/HT, transfected with chimeric transferase gt-HT; GT1-6/HT + HT1-8/GT, co-transfected with both chimeric transferases gt-HT and ht-GT; HT1-8/GT, transfected with chimeric transferase ht-GT; HT, transfected with normal HT; GT + HT, co-transfected with both normal transferases GT and HT. Blots were probed with a cDNA encoding GT (Top panel), HT (Middle panel) or g-actin (Bottom panel).

Figure 4. Enzyme kinetics of normal and chimeric glycosyltransferases

Lineweaver-Burk plots for α(1,3) galactosyltransferase (□) and α(1,2)fucosyltransferase (■) to determine the apparent Km values for N-acetyl lactosamine. Experiments were performed in triplicate, plots shown are of mean values of enzyme activity of wild-type transferases, GT and HT, and chimeric proteins ht-GT and gt-HT in transfected COS cell extracts using phenyl-B-D Gal and N-acetyl lactosamine as acceptor substrates.

Figure 5. Staining of cells co-transfected with chimeric transferases

Cells were co-transfected with cDNAs encoding

normal transferases GT + HT (panels A, B), with chimeric transferases gt-HT + ht-GT (panels C, D), with HT + ht-GT (panels E, F) or with GT + gt-HT (panels G, H) and 48h later were stained with FITC-labelled lectin IB4 (panels A, C, E, G) or UEAI (panels B, D, F, H).

Figure 6 is a representation of the nucleic acid sequence and corresponding amino acid sequence of pig secretor.

Figure 7 is a representation of the nucleic acid sequence and corresponding amino acid sequence of pig H.

Figure 8 Cell surface staining of pig endothelial cell line (PIEC) transfected with chimeric α(1,2)-fucosyltransferase. Cells were transfected and clones exhibiting stable integration were stained with UEAI lectin and visualised by fluorescence microscopy.

Figure 9 Screening of chimeric α(1,2)-fucosyltransferase transferase in mice. Mice were injected with chimeric α(1,2)-fucosyltransferase and the presence of the transferase was analysed by dot blots.

Description of the Preferred Embodiment

The nucleic acid sequences encoding the catalytic domain of a glycosyltransferase may be any nucleic acid sequence such as those described in PCT/US95/07554, which is herein incorporated by reference, provided that it encodes a functional catalytic domain with the desired glycosyltransferase activity.

Preferred catalytic domains from glycosyltransferase include H transferase and secretor. Preferably these are based on human or porcine sequences.

The nucleic acid sequences encoding the localisation signal of a second transglycosylase may be any nucleic acid sequence encoding a signal sequence such as signal sequences disclosed in P A Gleeson, R D Teasdale &

J Bourke, Targeting of proteins to the Golgi apparatus. Glycoconjugate J. (1994) 11: 381-394. Preferably the localisation signal is specific for the Golgi apparatus, more preferably for that of the trans Golgi. Still more preferably the localisation signal is based on that of Gal transferase. Even more preferably the localisation signal is based on porcine, murine or bovine sequences. Even more preferably the nucleic acid encodes a signal sequence with following amino acid sequence (in single letter code): MNVKGR (porcine), MNVKGK (mouse) or MVVKGK (bovine).

Vectors for expression of the chimeric enzyme may be any suitable vector, including those disclosed in PCT/US95/07554.

The nucleic acid of the invention can be used to produce cells and organs with the desired glycosylation pattern by standard techniques, such as those disclosed in PCT/US95/07554. For example, embryos may be transfected by standard techniques such as microinjection of the nucleic acid in a linear form into the embryo (22). The embryos are then used to produce live animals, the organs of which may be subsequently used as donor organs for implantation.

Cells, tissues and organs suitable for use in the invention will generally be mammalian cells. Examples of suitable cells and tissues such as endothelial cells, hepatic cells, pancreatic cells and the like are provided in PCT/US95/07554.

The invention will now be described with reference to the following non-limiting Examples.

ABBREVIATIONS

The abbreviations used are bp, base pair(s); FITC, fluorescein isothiocyanate; GT, galactosyltransferase; H substance, α(1,2)fucosyl lactosamine; HT, α(1,2)fucosyltransferase; PCR, polymerase chain reaction;

Example 1 Cytoplasmic domains of glycosyltransferases play a central role in the temporal action of enzymes

EXPERIMENTAL PROCEDURES

Plasmids - The plasmids used were prepared using standard techniques (7); pGT encodes the cDNA for the porcine α(1,3)galactosyltransferase (23), pHT encodes the cDNA for the α(1,2)fucosyltransferase (human) (25). Chimeric glycosyltransferase cDNAs were generated by polymerase chain reaction as follows: an 1105 bp product ht-GT was generated using primers corresponding to the 5′ end of ht-GT (5′-GCGGATCCATGTGGCTCCGGAGCC ATCGTCAGGTGGTTCTGTCAATGC TGCTTG-3′) coding for nucleotides 1-24 of HT (25) followed immediately by nucleotides 68-89 of GT (8) and containing a BamH1 site (underlined) and a primer corresponding to the 3′ end of ht-GT (5′-GCTCTAGAGCGTCAGATGTTATT TCTAACCAAATTATAC-3′) containing complementarity to nucleotides 1102-1127 of GT with an Xba1 site downstream of the translational stop site (underlined); an 1110 bp product gt-HT was generated using primers corresponding to the 5′ end of gt-HT (5′-GCGGATCCATGAATGTCAAAGGAAGACTCTGCCTGGCCT TCCTGC-3′) coding for nucleotides 49-67 of GT followed immediately by nucleotides 25-43 of HT and containing a BamH1 site (underlined) and a primer corresponding to the 3′ end of gt-HT (5′-GCTCTAGAGCCTCAAGGCTTAG CCAATGTCCAGAG-3′) containing complementarity to nucleotides 1075-1099 of HT with a Xba1 site downstream of the translational stop site (underlined). PCR products were restricted BamH1/Xba1, gel-purified and ligated into a BamH1/Xba1 digested pcDNA1

expression vector (Invitrogen) and resulted in two plasmids pht-GT (encoding the chimeric glycosyltransferase ht-GT) and pgt-HT (encoding the chimeric glycosyltransferase gt-HT) which were characterised by restriction mapping, Southern blotting and DNA sequencing .

Transfection and Serology - COS cells were maintained in Dulbecco's modified Eagles Medium (DMEM) (Trace Biosciences Pty. Ltd. , Castle Hill, NSW, Australia) and were transfected (1-10 µg DNA/5 x 105 cells) using DEAE-Dextran (26); 48h later cells were examined for cell surface expression of H substance or Gal-α(1,3)-Gal using FITC-conjugated lectins: IB4 lectin isolated from Griffonia simplicifolia (Sigma, St. Louis, MO) detects Gal-α(1,3)-Gal (27); UEAI lectin isolated from Ulex europaeus (Sigma, St. Louis, MO) detects H substance (28). H substance was also detected by indirect immunofluorescence using a monoclonal antibody (mAb) specific for the H substance (ASH-1952) developed at the Austin Research Institute, using FITC-conjugated goat anti-mouse IgG (Zymed Laboratories, San Francisco, CA) to detect mAb binding. Fluorescence was detected by microscopy.

RNA Analyses - Cytoplasmic RNA was prepared from transfected COS cells using RNAzol (Biotecx Laboratories, Houston, TX), and total RNA was electrophoresed in a 1% agarose gel containing formaldehyde, the gel blotted onto a nylon membrane and probed with random primed GT or HT cDNA.

Glycosyltransferase assays - Forty-eight hours after transfection, cells were washed twice with phosphate buffered saline and lysed in 1% Triton X-100/ 100 mM cacodylate pH 6. 5/ 25 mM MnCl2, at 4°C for 30 min; lysates were centrifuged and the supernatant collected and stored at -70°C. Protein concentration was determined by the Bradford method using bovine serum albumin as standard (29). Assays for HT activity (30) were performed in 25 µl containing 3mM [GDP-^{14}C]fucose (specific activity 287 mCi/mmol, Amersham International), 5mM ATP, 50mM MOPS pH 6. 5, 20 mM MnCl2, using 2-10 µl of cell extract

(approximately 15-20μg of protein) and a range of concentrations (7. 5 -75 mM) of the acceptor phenyl-B-D-galactoside (Sigma). Samples were incubated for 2h at 37°C and reactions terminated by the addition of ethanol and water. The amount of ^{14}C-fucose incorporated was counted after separation from unincorporated label using Sep-Pak C18 cartridges (Waters-Millipore, Millford, MA). GT assays (31) were performed in a volume of 25 μl using 3mM UDP[^{3}H]-Gal (specific activity 189mCi/mmol, Amersham International), 5mM ATP, 100mM cacodylate pH 6. 5, 20mM $MnCl_2$ and various concentrations (1 -10 mM) of the acceptor N-acetyl lactosamine (Sigma). Samples were incubated for 2h at 37°C and the reactions terminated by the addition of ethanol and water. ^{3}H-Gal incorporation was counted after separation from non-incorporated UDP[^{3}H]-Gal using Dowex I anion exchange columns (BDH Ltd. , Poole, UK) or Sep-Pak Accell plus QMA anion exchange cartridges (Waters-Millipore, Millford, MA). All assays were performed in duplicate and additional reactions were performed in the absence of added acceptor molecules, to allow for the calculation of specific incorporation of radioactivity.

RESULTS

<u>Expression of chimeric α(1,3)galactosylransferase and α(1,2)fucosylransferase cDNAs</u>

We had previously shown that when cDNAs encoding α(1,3)galactosylransferase (GT) and α(1,2)fucosyltransferase (HT) were transfected separately they could both function efficiently leading to expression of the appropriate carbohydrates: Gal-α(1,3)-Gal for GT and H substance for HT (32). However when the cDNAs for GT and HT were transfected together, the HT appeared to "dominate" over the GT in that H substance expression was normal, but Gal-α(1,3)-Gal was reduced. We excluded trivial reasons for this effect and considered that the localisation of the enzymes may be the reason. Thus, if the HT localisation signal placed the enzyme in an earlier temporal compartment

than GT, it would have "first use" of the N-acetyl lactosamine substrate. However, such a "first use" if it occurred, was not sufficient to adequately reduce GT. Two chimeric glycosyltransferases were constructed using PCR wherein the cytoplasmic tails of GT and HT were switched. The two chimeras constructed are shown in Fig.1: ht-GT which consisted of the NH_2 terminal cytoplasmic tail of HT attached to the transmembrane, stem and catalytic domains of GT; and gt-HT which consisted of the NH_2 terminal cytoplasmic tail of GT attached to the transmembrane, stem and catalytic domains of HT. The chimeric cDNAs were subcloned into the eukaryotic expression vector pcDNAI and used in transfection experiments.

The chimeric cDNAs encoding ht-GT and gt-HT were initially evaluated for their ability to induce glycosyltransferase expression in COS cells, as measured by the surface expression of the appropriate sugar using lectins. Forty-eight hours after transfection COS cells were tested by immunofluorescence for their expression of Gal-α(1,3)-Gal or H substance (Table 1 & Fig. 2). The staining with IB4 (lectin specific for Gal-α(1,3)-Gal) in cells expressing the chimera ht-GT (30% of cells stained positive) was indistinguishable from that of the normal GT staining (30%) (Table 1 & Fig. 2). Similarly the intense cell surface fluorescence seen with UEAI staining (the lectin specific for H substance) in cells expressing gt-HT (50%) was similar to that seen in cells expressing wild-type pHT (50%) (Table 1 & Fig. 2). Furthermore, similar levels of mRNA expression of the glycosyltransferases GT and HT and chimeric glycosyltransferases ht-GT and gt-HT were seen in Northern blots of total RNA isolated from transfected cells (Fig. 3). Thus both chimeric glycosyltransferases are efficiently expressed in COS cells and are functional indeed there was no detectable difference between the chimeric and normal glycosyltransferases.

Glycosyltransferase activity in cells transfected with chimeric cDNAs encoding ht-GT and gt-HT

To determine whether switching the cytoplasmic tails of GT and HT altered the kinetics of enzyme function, we compared the enzymatic activity of the chimeric glycosyltransferases with those of the normal enzymes in COS cells after transfection of the relevant cDNAs. By making extracts from transfected COS cells and performing GT or HT enzyme assays we found that N-acetyl lactosamine was galactosylated by both GT and the chimeric enzyme ht-GT (Fig 4. panel A) over a the 1-5mM range of substrate concentrations. Lineweaver-Burk plots showed that both GT and ht-GT have a similar apparent Michealis-Menten constant of Km 2. 6mM for N-acetyl lactosamine (Fig. 4. panel B). Further HT, and the chimeric enzyme gt-HT were both able to fucosylate phenyl-B-D-galactoside over a range of concentrations (7. 5 - 25 mM) (Fig. 4 panel C) with a similar Km of 2. 3mM (Fig. 4 panel D), in agreement with the reported Km of 2. 4mM for HT (25). Therefore the chimeric glycosyltransferases ht-GT and gt-HT are able to utilise N-acetyl lactosamine (ht-GT) and phenyl-B-D-galactoside (gt-HT) in the same way as the normal glycosyltransferases, thus switching the cytoplasmic domains of GT and HT does not alter the function of these glycosyltransferases and if indeed the cytoplasmic tail is the localisation signal then both enzymes function as well with the GT signal as with the HT signal.

Switching cytoplasmic domains of GT and HT results in a reversal of the "dominance" of the glycosyltransferases

The cDNAs encoding the chimeric transferases or normal transferases were simultaneously co-transfected into COS cells and after 48h the cells were stained with either IB4 or UEA1 lectin to detect Gal-α(1,3)-Gal and H substance respectively on the cell surface (Table 1 & Fig. 5). COS cells co-transfected with cDNAs for ht-GT + gt-HT (Fig 5 panel C) showed 30 % cells staining positive with IB4

(Table 1) but no staining on cells co-transfected with cDNAs for GT + HT (3%) (Fig. 5 panel A). Furthermore staining for H substance on the surface of ht-GT + gt-HT co-transfectants gave very few cells staining positive (5%) (Fig 5 panel D) compared to the staining seen in cells co-transfected with cDNAs for the normal transferases GT + HT (50%) (Fig. 5 panel B), ie. the expression of Gal-α(1,3)-Gal now dominates over that of H. Clearly, switching the cytoplasmic tails of GT and HT led to a complete reversal in the glycosylation pattern seen with the normal transferases i.e. the cytoplasmic tail sequences dictate the pattern of carbohydrate expression observed.

That exchanging the cytoplasmic tails of GT and HT reverses the dominance of the carbohydrate epitopes points to the glycosyltransferases being relocalized within the Golgi. To address this question, experiments were performed with cDNAs encoding glycosyltransferases with the same cytoplasmic tail: COS cells transfected with cDNAs encoding HT + ht-GT stained strongly with both UEAI (50%) and IB4 (30%) (Table 1 & Fig. 5 panels E, F), the difference in staining reflecting differences in transfection efficiency of the cDNAs. Similarly cells transfected with cDNAs encoding GT + gt-HT also stained positive with UEAI (50%) and IB4 (30%) (Table 1 & Fig. 5 panel G, H). Thus, glycosyltransferases with the same cytoplasmic tail leads to equal cell surface expression of the carbohydrate epitopes, with no "dominance" of one glycosyltransferase over the other observed, and presumably the glycosyltransferases localised at the same site appear to compete equally for the substrate.

In COS cells the levels of transcription of the cDNAs of chimeric and normal glycosyltransferases were essentially the same (Fig.3) and the immunofluorescence pattern of COS cells expressing the chimeric glycosyltransferases ht-GT and gt-HT showed the typical staining pattern of the cell surface Gal-α(1,3)-Gal and H substance respectively (Table 1 & Fig. 2), the pattern

being indistinguishable from that of COS cells expressing normal GT and HT. Our studies showed that the Km of ht-GT for N-acetyl lactosamine was identical to the Km of GT for this substrate, similarly the Km of gt-HT for phenylBDgalactoside was approximately the same as the Km of HT for phenylbDgalactoside (Fig. 3). These findings indicate that the chimeric enzymes are functioning in a cytoplasmic tail-independent manner, such that the catalytic domains are entirely functional, and are in agreement with those of Henion et al (23), who showed that an NH_2 terminal truncated marmoset GT (including truncation of the cytoplasmic and transmembrane domains) maintained catalytic activity and confirmed that GT activity is indeed independent of the cytoplasmic domain sequence.

If the Golgi localisation signal for GT and HT is contained entirely within the cytoplasmic domains of the enzymes, then switching the cytoplasmic tails between the two transferases should allow a reversal of the order of glycosylation. Co-transfection of COS cells with cDNA encoding the chimeric glycosyltransferases ht-GT and gt-HT caused a reversal of staining observed with the wild type glycosyltransferases (Fig. 5), demonstrating that the order of glycosylation has been altered by exchanging the cytoplasmic tails. Furthermore, co-transfection with cDNA encoding glycosyltransferases with the same cytoplasmic tails (i. e. HT + ht-GT and GT + gt-HT) gave rise to equal expression of both Gal-α(1,3)-Gal and H substance (Fig.5). The results imply that the cytoplasmic tails of GT and HT are sufficient for the localisation and retention of these two enzymes within the Golgi.

To date only twenty or so of at least one hundred predicted glycosyltransferases have been cloned and few of these have been studied with respect to their Golgi localisation and retention signals (34). Studies using the elongation transferase N-acetylglucosaminyltransferase I (33-37), the terminal transferases α(2,6)sialyltransferase (24-26) and β(1,4)galactosyltransferase (38-40) point to

residues contained within the cytoplasmic tail, transmembrane and flanking stem regions as being critical for Golgi localisation and retention. There are several examples of localisation signals existing within cytoplasmic tail domains of proteins including the KDEL and KKXX motifs in proteins resident within the endoplasmic reticulum (41,42) the latter motif also having been identified in the cis Golgi resident protein ERGIC-53 (43) and a di-leucine containing peptide motif in the mannose-6-phosphate receptor which directs the receptor from the trans-Golgi network to endosomes (44). These motifs are not present within the cytoplasmic tail sequences of HT or GT or in any other reported glycosyltransferase. To date a localisation signal in Golgi resident glycosyltransferases has not been identified and while there is consensus that transmembrane domains are important in Golgi localisation, it is apparent that this domain is not essential for the localisation of all glycosyltransferases, as shown by the study of Munro (45) where replacement of the transmembrane domain of α(2,6)sialyltransferase in a hybrid protein with a poly-leucine tract resulted in normal Golgi retention. Dahdal and Colley (46) also showed that sequences in the transmembrane domain were not essential to Golgi retention. This study is the first to identify sequence requirements for the localisation of α(1,2)fucosyltransferase and α(1,3)galactosyltransferase within the Golgi. It is anticipated that other glycosyltransferases will have similar localisation mechanisms.

Example 2 Use of secretor in construction of a chimeric enzyme

A construct is made using PCR and subcloning as described in Example 1, such that amino acids #1 to #6 of the pig α(1,3)-galactosyltransferase (MNVKGR) replace amino acids #1 to 5 of the pig secretor (Fig 6). Constructs are tested as described in Example 1.

<u>Example 3</u> <u>Use of pig H transferase in construction of a chimeric enzyme</u>

A construct is made using PCR and subcloning as described in Example 1, such that amino acids #1 to #6 of the pig α(1,3)-galactosyltransferase (MNVKGR) replace amino acids #1 to 8 of the pig H transferase (Fig 7). Constructs are tested as described in Example 1.

<u>Example 4</u>. <u>Generation of pig endothelial cells expressing chimeric α(1,2)fucosyltransferase</u>

The pig endothelial cell line PIEC expressing the chimeric α1,2fucosyltransferase was produced by lipofectamine transfection of pgtHT plasmid DNA (20 μg) and pSV2NEO (2 μg) and selecting for stable integration by growing the transfected PIEC in media containing G418 (500 μg/ml; Gibco-BRL, Gaithersburg, MD). Fourteen independant clones were examined for cell surface expression of H substance by staining with UEA-1 lectin. >95% of cells of each of these clones were found to be positive. Fig. 8 shows a typical FACS profile obtained for these clones.

<u>Example 5</u> <u>Production of transgenic mice expressing chimeric α(1,2)fucosyltransferase</u>

A NruI/NotI DNA fragment, encoding the full length chimeric α1,2fucosyltransferase, was generated utilising the Polymerase Chain Reaction and the phHT plasmid using the primers:

5' primer homologous to the 5'UTR:

5'-T<u>TCGCGA</u>ATGAATGTCAAAGGAAGACTCTG, in which the underlined sequence contains a unique NruI site;

3' primer homologous to the 3'UTR:

5'-G<u>GCGGCCGC</u>TCAGATGTTATTTCTAACCAAAT

the underlined sequence contains a NotI site

The DNA was purified on gels, electroeluted and subcloned into a NruI/NotI cut genomic H-2Kb containing vector resulting in the plasmid clone (pH-2Kb-gtHT)

encoding the chimeric α(1,2)-fucosyltransferase gene directionally cloned into exon 1 of the murine H-2Kb gene, resulting in a transcript that commences at the H-2Kb transcriptional start site, continuing through the gtHT cDNA insert. The construct was engineered such that translation would begin at the initiation codon (ATG) of the hHT cDNA and terminate at the in-phase stop codon (TGA).

DNA was prepared for microinjection by digesting pH-2Kb-hHT with XhoI and purification of the H-2Kb-hHT DNA from vector by electrophoretic separation in agarose gels, followed by extraction with chloroform, and precipitation in ethanol to decontaminate the DNA. Injections were performed into the pronuclear membrane of (C57BL/6xSJL)F1 zygotes at concentrations between 2-5ng/ml, and the zygotes transferred to pseudopregnant (C57BL/6xSJL)F1 females.

The presence of the transgene in the live offspring was detected by dot blotting. 5mg of genomic DNA was transferred to nylon filters and hybridized with the insert from gtHT, using a final wash at 68°C in 0.1xSSC/1% SDS. Fig. 9 shows the results of testing 12 live offspring, with two mice having the transgenic construct integrated into the genome. Expression of transgenic protein is examined by estimating the amount of UEAI lectin (specific for H substance) or anti-H mAb required to haemagglutinate red blood cells from transgenic mice. Hemagglutination in this assay demonstrates transgene expression.

It will be apparent to the person skilled in the art that while the invention has been described in some detail for the purposes of clarity and understanding, various modifications and alterations to the embodiments and methods described herein may be made without departing from the scope of the inventive concept disclosed in this specification.

References cited herein are listed on the following pages, and are incorporated herein by this

reference.

TABLE 1

EXPRESSION OF GAL-α(1,3)GAL AND H SUBSTANCE BY COS CELLS TRANSFECTED WITH cDNAs ENCODING NORMAL AND CHIMERIC GLYCOSYLTRANSFERASES

COS cells transfected with cDNA encoding:	%IB4 positive cells	%UEAI positive cells
GT	30	0
HT	0	50
ht-GT	30	0
gt-HT	3	50
GT+HT	3	50
ht-GT+gt-HT	33	5
GT+gt-HT	30	30
GT+ht-GT	30	0
HT+ht-GT	30	30
HT+gt-HT	0	50
Mock	0	0

Transfected COS cells were stained with FITC-labelled IB4 (lectin specific for Gal-α(1,3)Gal or UEAI (lectin specific for H substance) and positive staining cells were visualized and counted by fluorescence microscopy. Results are from at least three replicates.

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CLAIMS

1. A nucleic acid encoding a chimeric enzyme, wherein said chimeric enzyme comprises a catalytic domain of a first glycosyltransferase and a localisation signal of a second glycosyltransferase, whereby when said nucleic acid is expressed in a cell said chimeric enzyme is located in an area of the cell where it is able to compete for substrate with a second glycosyltransferase, resulting in reduced levels of a product from said second glycosyltransferase.

2. A nucleic acid according to claim 1, wherein said localisation signal localises said catalytic domain thereby to enable the catalytic domain to compete with said second glycosyltransferase for a substrate.

3. A nucleic acid according to claim 1 or claim 2, wherein the localisation signal is derived from a glycosyltransferase which produces glycosylation patterns which are recognised as foreign by a transplant recipient.

4. A nucleic acid according to any one of claims 1 to 3, wherein the localisation signal comprises the amino terminus of the second glycosyltransferase.

5. A nucleic acid according to any one of claims 1 to 4, wherein the localisation signal is derived from $\alpha(1,3)$-galactosyltransferase.

6. A nucleic acid according to any one of claims 1 to 5, wherein the first glycosyltransferase is selected from the group consisting of H-transferase, secretor sialyltransferase, a galactosyl sulphating enzyme or a phosphorylating enzyme.

7. A nucleic acid according to any one of claims 1 to 6, wherein the catalytic domain and the localisation signal each originates from a mammal selected from the group consisting of human, primates, ungulates, dogs, mice, rats and rabbits.

8. A nucleic acid according to any one of claims 1 to 7, wherein the localisation signal is derived from the

same species as the cell which the nucleic acid is intended to transform.

9. A nucleic acid according to any one of claims 1 to 8, comprising a sequence encoding the catalytic domain of H transferase and a nucleic acid sequence encoding a localisation signal from Gal transferase.

10. A nucleic acid according to claim 9, wherein the catalytic domain and the localisation signal are derived from pigs.

11. A nucleic acid according to any one of claims 1 to 10, which encodes gtHT as defined herein.

12. A vehicle comprising a nucleic acid according to any one of claims 1 to 11.

13. vehicle according to claim 12, selected from the group consisting of an expression vector, plasmid and phage.

14. A vehicle according to claim 12 or claim 13, which enables said nucleic acid to be expressed in prokaryotes or in eukaryotes.

15. An isolated nucleic acid molecule encoding a localisation signal of a glycosyltransferase.

16. An isolated nucleic acid molecule according to claim 15, wherein the signal encoded comprises an amino terminus of gal-transferase.

17. A method of producing a nucleic acid according to any one of claims 1 to 11, comprising the step of operably linking a nucleic acid sequence encoding a catalytic domain from a first glycosyltransferase to a nucleic acid sequence encoding a localisation signal of a second glycosyltransferase.

18. A method of reducing the level of a carbohydrate exhibited on the surface of a cell, said method comprising causing a nucleic acid to be expressed in said cell wherein said nucleic acid encodes a chimeric enzyme which comprises a catalytic domain of a first glycosyltransferase and a localisation signal of a second glycosyltransferase, whereby said chimeric enzyme is located in an area of the

cell where it is able to compete for substrate with said second glycosyltransferase, and wherein said second glycosyltransferase is capable of producing said carbohydrate.

19. A method of producing a cell from a donor species which is immunologically acceptable to a recipient species by reducing levels of carbohydrate on said cell which cause it to be recognised as non-self by the recipient, said method comprising causing a nucleic acid to be expressed in said cell wherein said nucleic acid encodes a chimeric enzyme which comprises a catalytic domain of a first glycosyltransferase and a localisation signal of a second glycosyltransferase, whereby said chimeric enzyme is located in an area of the cell where it is able to compete for substrate with said second glycosyltransferase, and wherein said second glycosyltransferase is capable of producing said carbohydrate.

20. A cell produced by a method according to claim 19.

21. An organ comprising a cell according to claim 20.

22. A non-human transgenic animal, organ or cell comprising the nucleic acid according to any one of claims 1 to 11.

23. An expression unit which expresses a nucleic acid according to any one of claims 1 to 11, resulting in a cell which is immunologically acceptable to an animal having reduced levels of a carbohydrate on its surface, which carbohydrate is recognised as non-self by said species.

24. An expression unit according to claim 23, selected from the group consisting of a retroviral-packaging cassette, retroviral construct or retroviral producer cell.

25. A method of producing an expression unit according to claim 23 or claim 24, said unit having reduced levels of a carbohydrate on its surface wherein the carbohydrate is recognised as non-self by a species, comprising transforming/transfecting a retroviral packaging

cell or a retroviral producer cell with the nucleic acid of the invention under conditions such that the chimeric enzyme is produced.

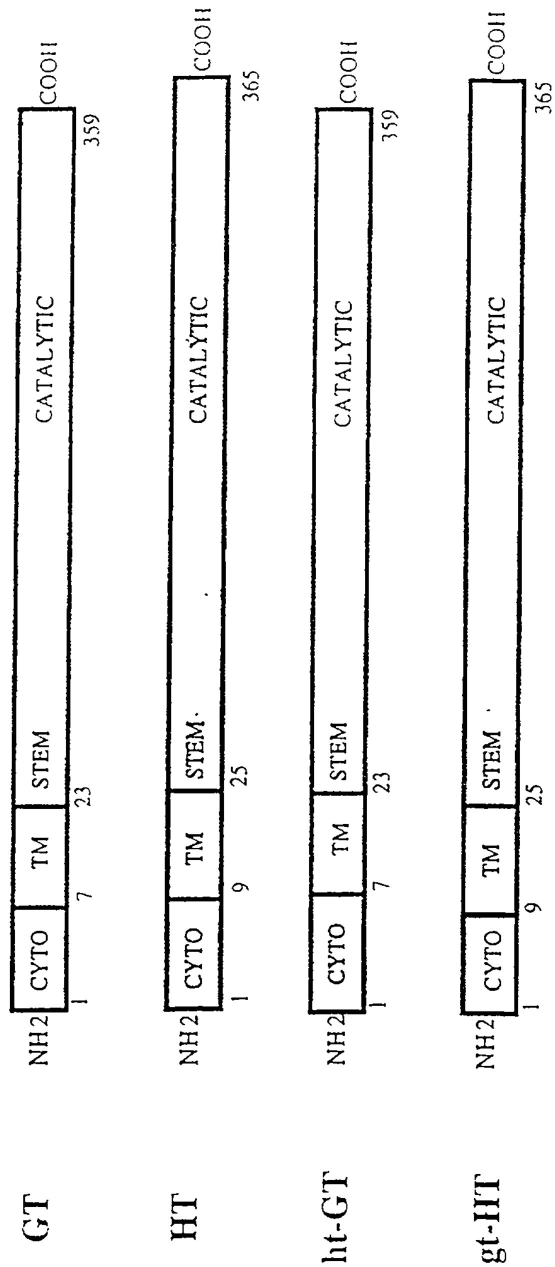
GT
NH2
CYTO
1
TM
7
STEM
23
CATALYTIC
359
COOH
HT
NH2
CYTO
1
TM
9
STEM
25
CATALYTIC
365
COOH
ht-GT
NH2
CYTO
1
TM
7
STEM
23
CATALYTIC
359
COOH
gt-HT
NH2
CYTO
1
TM
9
STEM
25
CATALYTIC
365
COOH

FIGURE 1

2/12

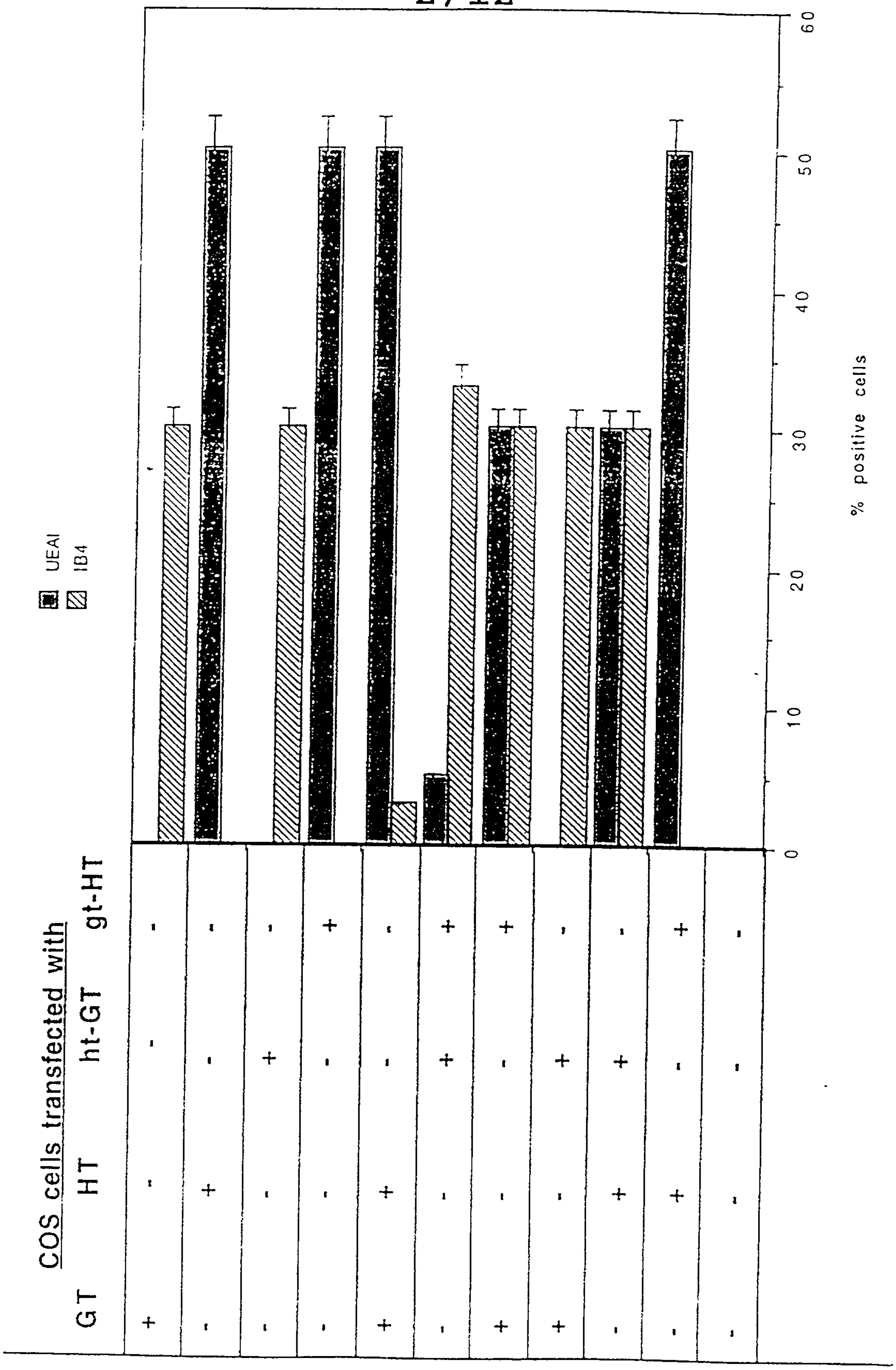
COS cells transfected with
GT
HT
ht-GT
gt-HT
UEAI
IB4
% positive cells
0
10
20
30
40
50
60

FIGURE 2

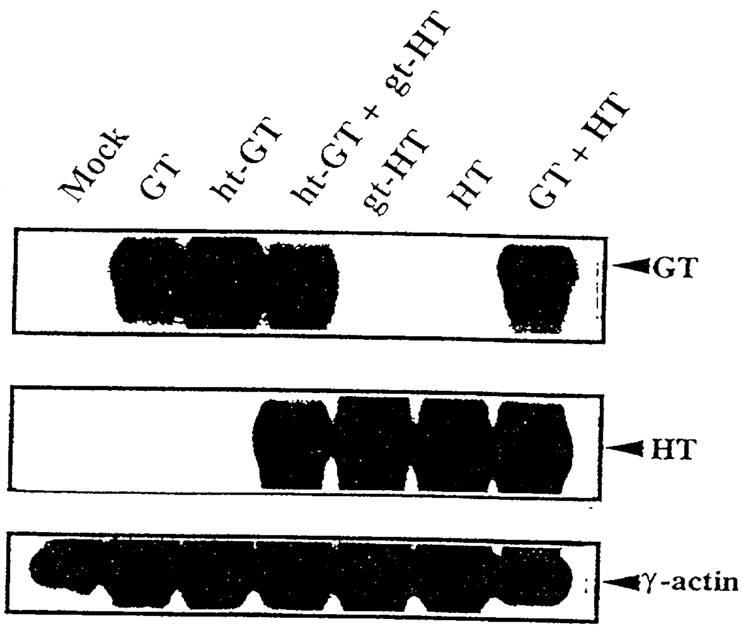

FIGURE 3

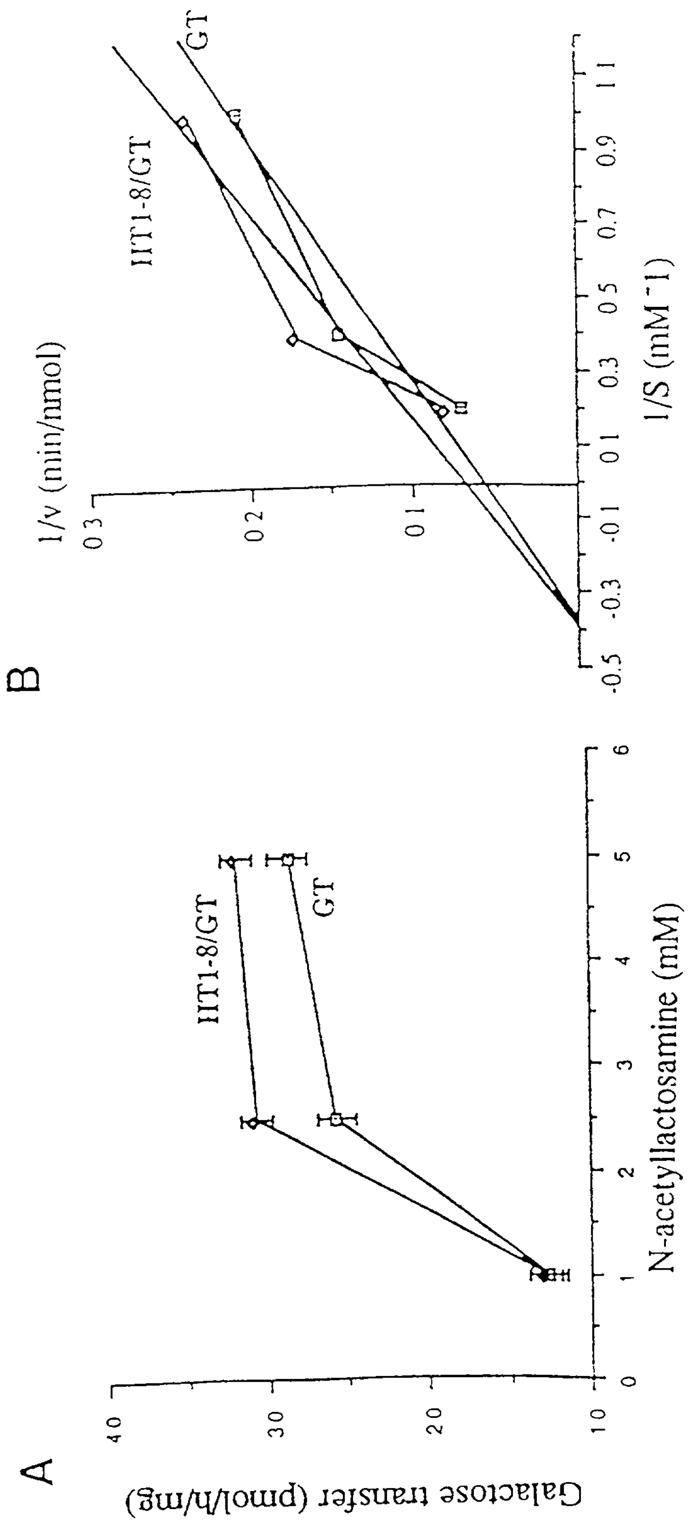
A
Galactose transfer (pmol/h/mg)
N-acetyllactosamine (mM)
IIT1-8/GT
GT
B
1/v (min/nmol)
1/S (mM⁻1)
IIT1-8/GT
GT

FIGURE 4

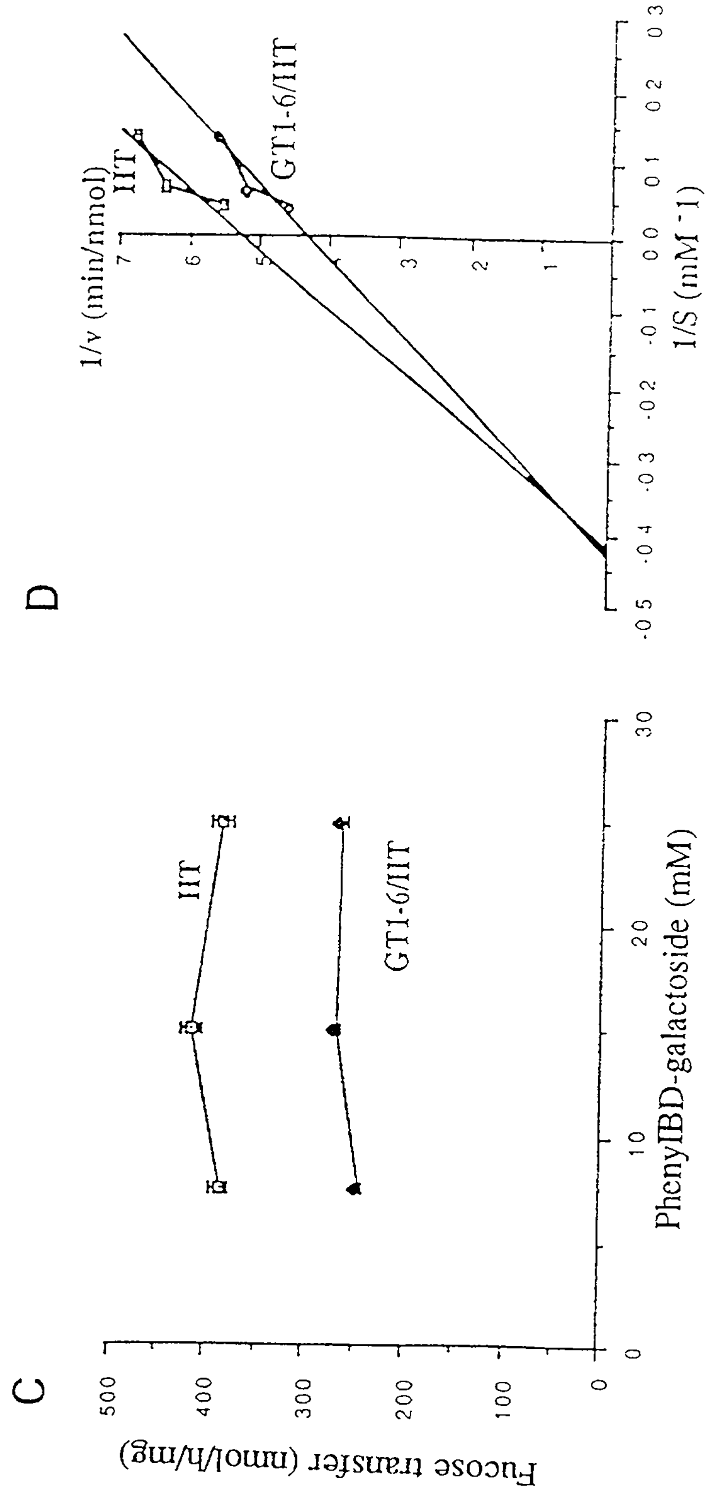

FIGURE 4 Continued

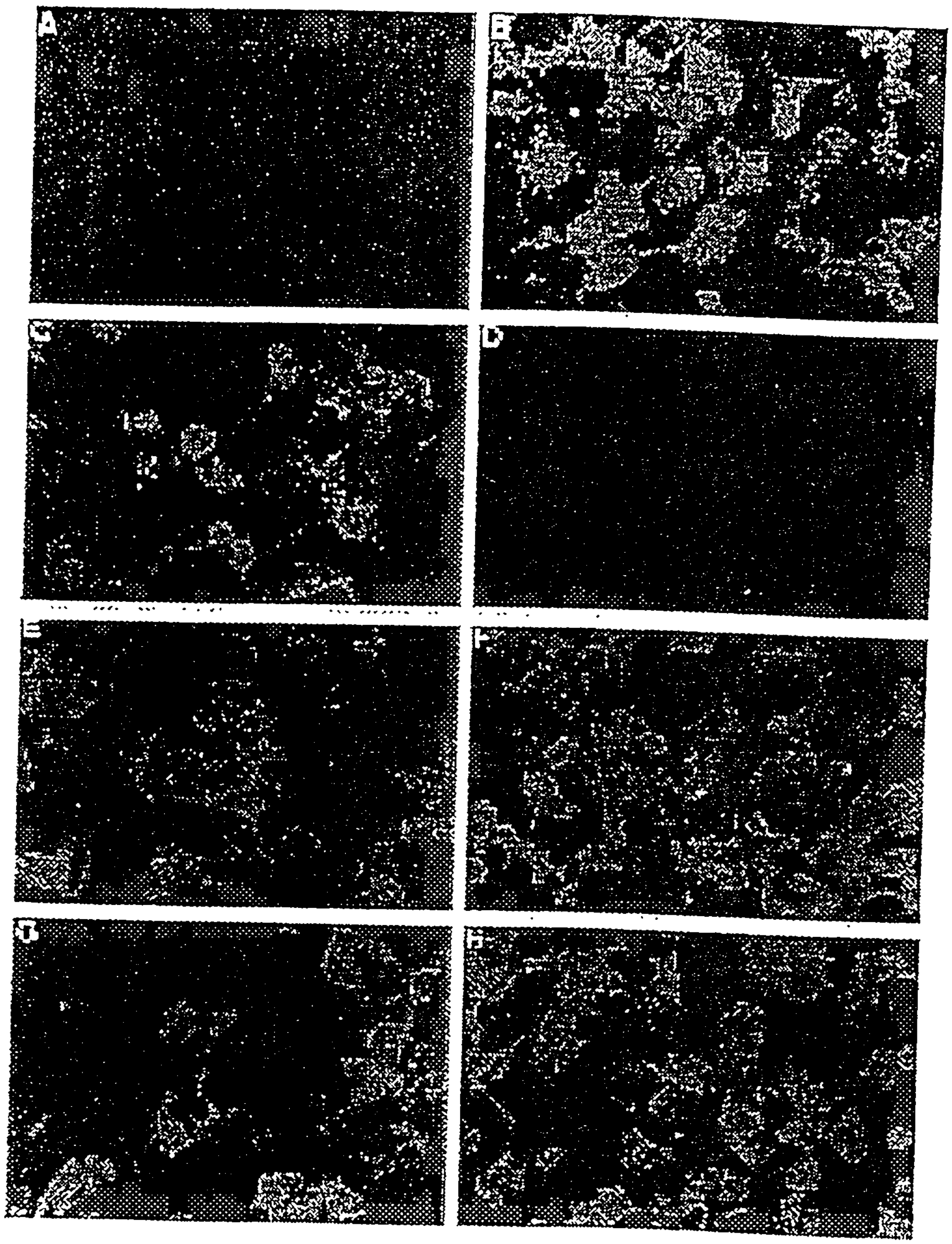

FIGURE 5

PORCINE SECRETOR SEQUENCE

```
             M   L   S   M   Q   A   S   F   F   F   P   T   G   P   F   I   L   17
CT  ACA GCC ATG CTC AGC ATG CAG GCA TCC TTC TTC TTC CCC ACG GGT CCC TTC ATC CTC  59

F   V   F   T   A   S   T   I   F   H   L   Q   Q   R   M   V   K   I   Q   P   37
TTT GTC TTC ACG GCT TCC ACC ATA TTT CAC CTT CAG CAG AGG ATG GTG AAG ATT CAA CCC 119

T   W   E   L   Q   M   V   T   Q   V   T   T   E   S   P   S   S   P   Q   L   57
ACG TGG GAG TTA CAG ATG GTG ACG CAG GTG ACC ACA GAG AGC CCC TCG AGC CCC CAG CTG 179
```

PORCINE SECRETOR SEQUENCE

```
K   G   M   W   T   I   N   A   I   G   R   L   G   N   Q   M   G   E   Y   A   77
AAG GGC ATG TGG ACG ATC AAT GCC ATC GGC CGC CTG GGG AAC CAG ATG GGG GAG TAC GCC 239

T   L   Y   A   L   A   R   M   N   G   R   P   A   F   I   P   P   E   M   H   97
ACC CTG TAC GCG CTG GCC AGG ATG AAC GGG CGG CCG GCC TTC ATC CCG CCC GAG ATG CAC 299

S   T   L   A   P   I   F   R   I   T   L   P   V   L   H   A   S   T   A   R   117
AGC ACG CTG GCC CCC ATC TTC AGG ATC ACC CTC CCG GTC CTG CAC GCC AGC ACG GCC CGC 359

R   I   P   W   Q   N   Y   H   L   N   D   W   M   E   E   R   Y   R   H   I   137
AGG ATC CCC TGG CAG AAC TAC CAC CTG AAC GAC TGG ATG GAG GAG CGG TAC CGC CAC ATC 419

P   G   E   Y   V   R   L   T   G   Y   P   C   S   W   T   F   Y   H   H   L   157
```

FIGURE 6

CCG GGG GAG TAC GTG CGC CTC ACG GGC TAC CCC TGC TCC TGG ACC TTC TAC CAC CAC CTG 479

R T E I L R E F T L H N H V R E E A Q D 177
CGC ACC GAG ATC CTC CGG GAG TTC ACC CTG CAT AAC CAC GTG CGC GAG GAG GCC CAG GAT 539

F L R G L R V N G S R P S T Y V G V H V 197
TTC CTG CGG GGT CTG CGG GTG AAC GGG AGC CGA CCG AGT ACC TAC GTG GGG GTG CAC GTG 599

R R G D Y V H V M P N V W K G V V A D R 217
CGC CGG GGG GAc TAC GTG CAC GTG ATG CCC AAC GTG TGG AAG GGC GTG GTG GCC GAC CGG 659

R Y L E Q A L D W F R A R Y R S P V F V 237
CGG TAC CTG GAG CAG GCC CTG GAC TGG TTC CGG GCT CGC TAC CGC TCC CCc GTC TTT GTG 719

V S S N G M A W C R E N I N A S R G D V 257
GTC TCC AGC AAC GGC ATG GCC TGG TGT CGG GAA AAC ATC AAT GCC TCG CGC GGC GAT GTG 779

V F A G N G I E G S P A K D F A L L T Q 277
GTG TTT GCC GGC AAT GGC ATC GAG GGC TCC CCC GCC AAA GAC TTC GCG CTG CTC ACG CAG 839

C N H T V M T I G T F G I W A A Y L A G 297
TGT AAC CAC ACT GTC ATG ACC ATT GGC ACG TTC GGG ATc TGG GCC GCC TAC CTT GCT GGT 899

G E T I Y L A N Y T L P D S P F L K L F 317
GGA GAG ACC ATC TAC CTG GCC AAT TAC ACG CTC CCG GAC TCT CCC TTC CTC AAA CTC TTT 959

FIGURE 6 (cont.)

```
K   P   E   A   A   F   L   P   E   W   I   G   I   E   A   D   L   S   P   L    337
AAG CCC GAG GCA GCC TTC CTG CCC GAG TGG ATT GGG ATC GAG GCA GAC CTG TCC CCA CTC  1019

L   K   H   *                                                   340
CTT AAG CAC TGA TGT CGG CTG TCC                                         1043
```

FIGURE 6 (cont.)

10/12

PIG H TRANSFERASE

M W V P S P R H L C L T F L- L V C V L A 20
ATGTGGGTCCCCAGCCCGCCCCACCTCTGTCTGACCTTCCTGCTAGTCTGTGTTTTAGCA 60

A I F F L N V Y Q D L F Y S G L D L L A 40
GCAATTTTCTTCCTGAACGTCTATCAAGACCTCTTTTACAGTGGCTTAGACCTGCTGGCC 120

L C P D H N V V S S P V A I F C L A G T 60
CTGTGTCCAGACCATAACGTGGTATCATCTCCCGTGGCCATATTCTGCCTGGCGGGCACG 180

P V H P N A S D S C P K H P A S F S G T 80
CCGGTACACCCCAACGCCTCCGATTCCTGTCCCAAGCATCCTGCCTCCTTTTCCGGGACC 240

W T I Y P D G R F G N Q M G Q Y A T L L 100
TGGACTATTTACCCGGATGGCCGGTTTGGGAACCAGATGGGACAGTATGCCACGCTGCTG 300

A L A Q L N G R Q A F I Q P A M H A V L 120
GCCCTGGCGCAGCTCAACGGCCGCCAGGCCTTCATCCAGCCTGCCATGCACGCCGTCCTG 360

A P V F-R I T L P V L A P E V D R H A P 140
GCCCCCGTGTTCCGCATCACGCTGCCTGTCCTGGCCCCCGAGGTAGACAGGCACGCTCCT 420

W R E L E L H D W M S E D Y A H L K E P 160
TGGCGGGAGCTGGAGCTTCACGACTGGATGTCCGAGGATTATGCCCACTTAAAGGAGCCC 480

W L K L T G F P C S W T F F H H L R E Q 180
TGGCTGAAGCTCACCGGCTTCCCCTGCTCCTGGACCTTCTTCCACCACCTCCGGGAGCAG 540

I R S E F T L H D H L R Q E A Q G V L S 200
ATCCGCAGCGAGTTCACCCTGCACGACCACCTTCGGCAAGAGGCCCAGGGGGTACTGAGT 600

Q F R L P R T G D R P S T F V G V H V R 220
CAGTTCCGTCTACCCCGCACAGGGgACCGCCCCAGCACCTTCGTGGGGGTCCACGTGCGC 660

R G D Y L R V M P K R W K G V V G D G A 240
CGCGGGGACTATCTGCGTGTGATGCCCAAGCGCTGGAAGGGGGTGGTGGGTGACGGCGCT 720

Y L Q Q A M D W F R A R Y E A P V F V V 260
TACCTCCAGCAGGCTATGGACTGGTTCCGGGCCCGATACGAAGCCCCCGTCTTTGTGGTC 780

T S N G M E W C R K N I D T S R G D V I 280
ACCAGCAACGGCATGGAGTGGTGCCGGAAGAACATCGACACCTCCCGGGGGGACGTGATC 840

F A G D G R E A A P A R D F A L L V Q C 300
TTTGCTGGCGATGGGCGGGAGGCCGCGCCCGCCAGGGACTTTGCGCTGCTGGTGCAGTGC 900

N H T I M T I G T F G F W A A Y L A G G. 320
AACCACACCATCATGACCATTGGCACCTTCGGCTTCTGGGCCGCcTACCTGGCTGGTGgA 960

D T I Y L A N F T L P T S S F L K I F K 340
GATACcATCTACTTGGCTAACTTCACCCTGCCcACTTCCAGCTTCCTGAAGATCTTTAAA 1020

P E A A F L P E W V G I N A D L S P L Q 360
CcCGAGGCTGCCTTCCTGCCCGAGTGGGTGGGCATTAATGCAGACTTGTCTCCACTCCAG 1080

M L A G P * 365
ATGTTGGCTGGGCCTTGA 1098

FIGURE 7

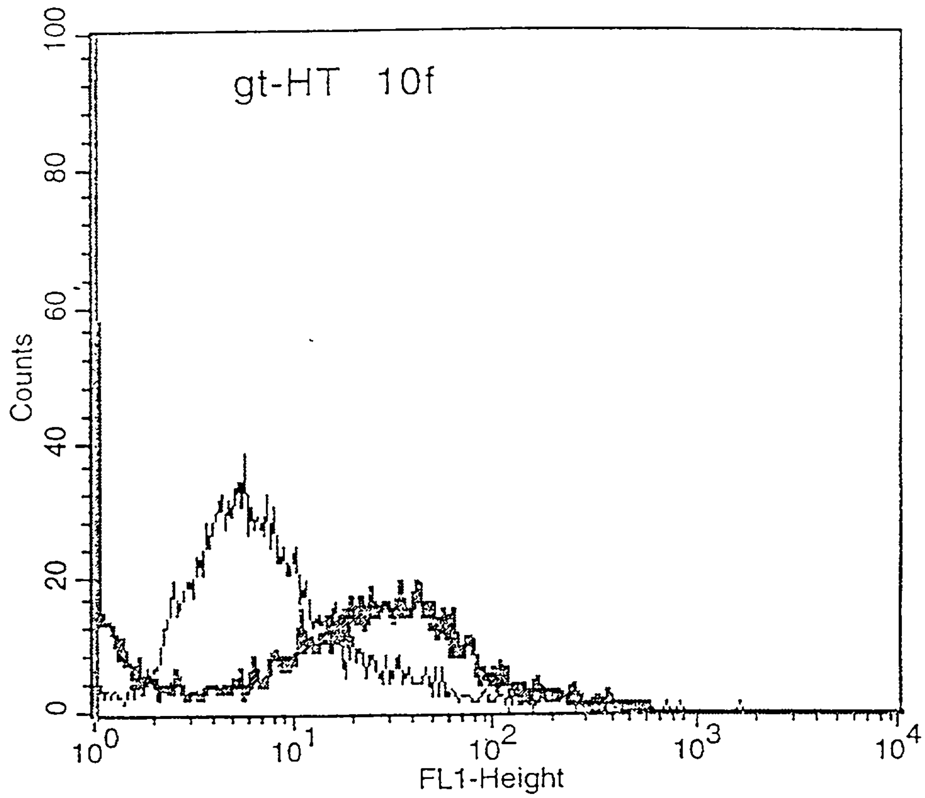

FIGURE 8

FIGURE 9

For Continuation-in-Part (CIP) Applications, complete

U.S. APPLICATION NUMBER	DATE OF FILING (day, month, year)	STATUS (patented, pending, abandoned)

I hereby appoint the following attorney(s) and/or patent agent(s) to prosecute this application and to transact all business in the Patent and Trademark Office connected herewith:

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Beard, John L.	Reg No 27,612	Golia, Charles E	Reg No 26,896	Reich, John C.	Reg No 37,703
Beck, Robert C	Reg. No 28,184	Gorman, Alan G	Reg No P-38,472	Reiland, Earl D	Reg. No 25,767
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Carlson, Alan G	Reg No 25,959	Hollingsworth, Mark A	Reg No 38,491	Smith, Jerome R	Reg No 35,684
Carter, Charles G	Reg No 35,093	Kastelic, Joseph M	Reg No 37,160	Sorensen, Andrew D	Reg No 33,606
Caspers, Philip P	Reg No 33,227	Kowalchyk, Alan W	Reg No 31,535	Stinebruner, Scott A	Reg No 38,323
Clifford, John A	Reg No 30,247	Kowalchyk, Katherine M	Reg No 36,848	Strawbridge, Douglas A	Reg No 28,376
Conrad, Timothy R	Reg No 30,164	Krull, Mark A	Reg No 34,205	Strodthoff, Kristine M	Reg No 34,259
Crawford, Robert	Reg No 32,122	Lacy, Paul A	Reg. No P-38,946	Sumner, John P	Reg No 29,114
Daignault, Ronald A	Reg No 25,968	Lasky, Michael B	Reg No 29,555	Sumners, John S	Reg No 24,216
Daley, Dennis R	Reg. No 34,994	Lynch, David W	Reg No 36,204	Tellekson, David K	Reg No 32,314
Daulton, Julie R	Reg No 36,414	Mau, Michael L	Reg No 30,087	Underhill, Albert L	Reg No 27,403
Dempster, Shawn B	Reg No 34,321	McDonald, Daniel W	Reg No 32,044	Vandenburgh, J Derek	Reg No 32,179
DiPietro, Mark J	Reg No 28,707	McDonald, Wendy M	Reg No 32,427	Vietzke, Lance L	Reg No 36,708
Edell, Robert T	Reg No 20,187	Mueller, Douglas P	Reg No 30,300	Welter, Paul A	Reg No 20,890
Farber, Michael B	Reg No 32,612	Nelson, Albin J	Reg No 28,650	Williams, Douglas J	Reg No 27,054
Fauver, Cole M	Reg No. 36,797			Wood, Gregory B	Reg No 28,133

68

I hereby authorize them to act and rely on instructions from and communicate directly with the person/assignee/attorney/firm/organization/who/which first sends/sent this case to them and by whom/which I hereby declare that I have consented after full disclosure to be represented unless/until I instruct Merchant & Gould to the contrary

Please direct all correspondence in this case to Merchant, Gould, Smith, Edell, Welter & Schmidt at the address indicated below (or if no address is specified, the first address):

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  Telephone No. (612) 332-5300
- 1000 Norwest Center, St. Paul, MN 55101-2701
  Telephone No. (612) 298-1055
- Suite 400, 11150 Santa Monica Boulevard, Los Angeles, CA 90025-3302
  Telephone No. (310) 445-1140

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Insert FULL name(s) AND address(es) of actual inventor(s)

201	FULL NAME OF INVENTOR	FAMILY NAME MCKENZIE	FIRST GIVEN NAME Ian	SECOND GIVEN NAME Farquhar Campbell
	RESIDENCE & CITIZENSHIP	CITY BRUNSWICK A/U	STATE OR FOREIGN COUNTRY VICTORIA	COUNTRY OF CITIZENSHIP AUSTRALIA
	POST OFFICE ADDRESS	POST OFFICE ADDRESS 359 BRUNSWICK ROAD	CITY BRUNSWICK	STATE & ZIP CODE/COUNTRY VICTORIA 3056
202	FULL NAME OF INVENTOR	FAMILY NAME SANDRIN	FIRST GIVEN NAME Mauro	SECOND GIVEN NAME Sergio
	RESIDENCE & CITIZENSHIP	CITY BRUNSWICK A/U	STATE OR FOREIGN COUNTRY VICTORIA	COUNTRY OF CITIZENSHIP AUSTRALIA
	POST OFFICE ADDRESS	POST OFFICE ADDRESS 211 BARKLEY STREET	CITY BRUNSWICK	STATE & ZIP CODE/COUNTRY VICTORIA 3056
203	FULL NAME OF INVENTOR	FAMILY NAME	FIRST GIVEN NAME	SECOND GIVEN NAME
	RESIDENCE & CITIZENSHIP	CITY	STATE OR FOREIGN COUNTRY	COUNTRY OF CITIZENSHIP
	POST OFFICE ADDRESS	POST OFFICE ADDRESS	CITY	STATE & ZIP CODE/COUNTRY

1-00

2-00

Each inventor must sign & date

Note: No legalization or other witness required

SIGNATURE OF INVENTOR 201	SIGNATURE OF INVENTOR 202	SIGNATURE OF INVENTOR 203
[signature]	[signature]	
DATE 20/3/98	DATE 20/3/98	DATE

For Additional Inventors:

☐ Check box and attach sheet with same information, including date and signature.

Revised 12/09/94

§ 1.56 Duty to disclose information material to patentability.

(a) A patent by its very nature is affected with a public interest. The public interest is best served, and the most effective patent examination occurs when, at the time an application is being examined, the Office is aware of and evaluates the teachings of all information material to patentability. Each individual associated with the filing and prosecution of a patent application has a duty of candor and good faith in dealing with the Office, which includes a duty to disclose to the Office all information known to that individual to be material to patentability as defined in this section. The duty to disclose information exists with respect to each pending claim until the claim is cancelled or withdrawn from consideration, or the application becomes abandoned. Information material to the patentability of a claim that is cancelled or withdrawn from consideration need not be submitted if the information is not material to the patentability of any claim remaining under consideration in the application. There is no duty to submit information which is not material to the patentability of any existing claim. The duty to disclose all information known to be material to patentability is deemed to be satisfied if all information known to be material to patentability of any claim issued in a patent was cited by the Office or submitted to the Office in the manner prescribed by § 1.97(b)-(d) and 1.98. However, no patent will be granted on an application in connection with which fraud on the Office was practiced or attempted or the duty of disclosure was violated through bad faith or intentional misconduct. The Office encourages applicants to carefully examine:

(1) prior art cited in search reports of a foreign patent office in a counterpart application, and

(2) the closest information over which individuals associated with the filing or prosecution of a patent application believe any pending claim patentably defines, to make sure that any material information contained therein is disclosed to the Office.

(b) Under this section, information is material to patentability when it is not cumulative to information already of record or being made of record in the application, and

(1) It establishes, by itself or in combination with other information, a prima facie case of unpatentability of a claim; or

(2) It refutes, or is inconsistent with, a position the applicant takes in:

(i) Opposing an argument of unpatentability relied on by the Office, or

(ii) Asserting an argument of patentability.

A prima facie case of unpatentability is established when the information compels a conclusion that a claim is unpatentable under the preponderance of evidence, burden-of-proof standard, giving each term in the claim its broadest reasonable construction consistent with the specification, and before any consideration is given to evidence which may be submitted in an attempt to establish a contrary conclusion of patentability.

(c) Individuals associated with the filing or prosecution of a patent application within the meaning of this section are:

(1) Each inventor named in the application;

(2) Each attorney or agent who prepares or prosecutes the application; and

(3) Every other person who is substantively involved in the preparation or prosecution of the application and who is associated with the inventor, with the assignee or with anyone to whom there is an obligation to assign the application.

(d) Individuals other than the attorney, agent or inventor may comply with this section by disclosing information to the attorney, agent, or inventor.

For Utility, PCT, and Design Applications

▼ INSTRUCTIONS

MERCHANT & GOULD

United States Patent Application

COMBINED DECLARATION AND POWER OF ATTORNEY

As a below named inventor I hereby declare that: my residence, post office address and citizenship are as stated below next to my name; that

I verily believe I am the original, first and sole inventor (if only one name is listed below) or a joint inventor (if plural inventors are named below) of the subject matter which is claimed and for which a patent is sought on the invention entitled:

Insert TITLE of invention

IMPROVED NUCLEIC ACIDS ENCODING A CHIMERIC GLYCOSYLTRANSFERASE

Check a or b

The specification of which

a. ☐ is attached hereto

b. ☐ was filed on ________

If "b" checked, complete

as application serial no. ________

and was amended on ________ (if applicable)

If PCT Application

Insert Int. application number & filing date

(in the case of PCT-filed application)

described and claimed in international no. PCT/AU97/00492 filed 1 August 1997

and as amended on ________ (if any), which I have reviewed and for which I solicit a United States patent.

I hereby state that I have reviewed and understand the contents of the above identified specification, including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose information which is material to the examination of this application in accordance with Title 37, Code of Federal Regulations, § 1.56(a). (Reprinted on back side)

I hereby claim foreign priority benefits under Title 35, United States Code, § 119/365 of any foreign application(s) for patent of inventor's certificate listed below and have also identified below any foreign application for patent or inventor's certificate having a filing date before that of the application on the basis of which priority is claimed:

Prior applications

Check a or b

a. _ no such applications have been filed.

b ☒ such applications have been filed as follows:

If "b" checked, complete

FOREIGN APPLICATION(S), IF ANY, CLAIMING PRIORITY UNDER 35 USC § 119			
COUNTRY	APPLICATION NUMBER	DATE OF FILING (day, month, year)	DATE OF ISSUE (day, month, year)
AUSTRALIA	PO1402	2/8/1996	
UNITED STATES	60/024,279	21/8/1996	

ALL FOREIGN APPLICATIONS, IF ANY, FILED BEFORE THE PRIORITY APPLICATION(S)			
COUNTRY	APPLICATION NUMBER	DATE OF FILING (day, month, year)	DATE OF ISSUE (day, month, year)

I hereby claim the benefit under Title 35, United States Code, § 120/365 of any United States and PCT international application(s) listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States application in the manner provided by the first paragraph of Title 35, United States Code, § 112, I acknowledge the duty to disclose material information as defined in Title 37, Code of Federal Regulations, § 1.56(a) which occurred between the filing date of the prior application and the national or PCT international filing date of this application

Revised 12/09/94

Applicant or Patentee: THE AUSTIN RESEARCH INSTITUTE

Serial or Patent No:

Attorney's Docket No:

Filed or Issued:

For:

VERIFIED STATEMENT (DECLARATION)
CLAIMING SMALL ENTITY STATUS
(37 CFR 1.9(f) and 1.27(d)) - NONPROFIT ORGANIZATION

I hereby declare that I am an official empowered to act on behalf of the nonprofit organization identified below:

NAME OF ORGANIZATION: THE AUSTIN RESEARCH INSTITUTE

ADDRESS OF ORGANIZATION: Kronheimer Building
The Austin Hospital
Studley Road
HEIDELBERG VIC 3084
AUSTRALIA

TYPE OF ORGANIZATION:

[ ] University or other institution of higher education
[ ] Tax exempt under Internal Revenue Service Code (26 USC 501(a) and 501(c)(3))
[ ] Nonprofit scientific or educational under statute of state of The United States of America
(Name of state)
(Citation of statute)
[ ] Would qualify as tax exempt under Internal Revenue Service Code (26 USC 501(a) and 501(c)(3)) if located in The United States of America
[x] Would qualify as nonprofit scientific or educational under statute of state of The United States of America if located in The United States of America
(Name of state)
(Citation of statute)

I hereby declare that the nonprofit organization identified above qualifies as a nonprofit organization as defined in 37 CFR 1.9(e) for purposes of paying reduced fees under section 41(a) and (b) of Title 35, United States Code with regard to the invention entitled:

"IMPROVED NUCLEIC ACIDS ENCODING A CHIMERIC GLYCOSYLTRANSFERASE"

by inventor(s): Ian Farquhar Campbell MCKENZIE
Mauro Sergio SANDRIN

described in:

[ ] the specification filed herewith
[x] application serial no. PCT/AU97/00492, filed 1 August 1997
[ ] patent no. , issued

I hereby declare that rights under contract or law have been conveyed to and remain with the nonprofit organization with regard to the above identified invention.

If the rights held by the above identified nonprofit organization are not exclusive, each individual, concern or organization having rights to the invention is listed below * and no rights to the invention are held by any person, other than the inventor, who could not qualify as a small business concern under 37 CFR 1.9(d) or by any concern which would not qualify as a small business concern under 37 CFR 1.9(d) or a nonprofit organization under 37 CFR 1.9(e). * NOTE: Separate verified statements are required from each named person, concern or organization having rights to the invention averring to their status as small entities (37 CFR 1.27).

I acknowledge the duty to file, in this application or patent, notification of any change in status resulting in loss of entitlement to small entity status prior to paying, or at the time of paying, the earliest of the issue fee or any maintenance fee due after the date on which status as a small entity is no longer appropriate (37 CFR 1.28(b)).

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application, any patent issuing thereon, or any patent to which this verified statement is directed.

NAME OF PERSON SIGNING: Michael Vovos

TITLE IN ORGANIZATION: Business Manager

ADDRESS OF PERSON SIGNING: Kronheimer Building, The Austin Hospital, Studley Road
HEIDELBERG VIC 3084, AUSTRALIA

SIGNATURE

DATE: 20 March 1998